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Ultrastructural Localization of Phosphatase(s) in the Body Wall of *Angiostrongylus cantonensis*

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Introduction

Phosphatases have been generally detected histochemically in sites where absorption, secretion and excretion occur. In many parasitic helminths, much attention has been called to the localization of phosphatases in relation to absorption of nutrients (Lumsden, 1975).

Yanagisawa *et al.* (1970, 1973) studied the absorption sites of glucose in two species of blood nematodes, *Angiostrongylus cantonensis* and *Dirofilaria immitis*. They reported the possibility that these blood nematodes absorb glucose through the body wall on the basis of the results from experiments with ligated worms or from the autoradiographic study. And these experiments provide strong direct evidence in favour of the Lee's view (1965) that the cuticle of some nematodes, particularly of those inhabiting host body fluid, is permeable to water, nonelectrolytes, and certain ions. Yanagisawa *et al.* (1970, 1973) also made histochemical studies with these blood nematodes to demonstrate acid phosphatase in high concentration in the hypodermis (subcuticular layer). Furthermore Maki and Yanagisawa (1976, 1977) carried out biochemical studies using intact *A. cantonensis* and suggested that phosphatase in the body wall hydrolyzes various phosphomonoesters in the surrounding medium, for example, β-glycerophosphate (pH 5.0 and 7.3), adenosine-5′-monophosphate (pH 7.3) and glucose-1-phosphate (pH 5.0 and 7.3). The present study, which extends the work by Yanagisawa *et al.* (1970, 1973) and by Maki and Yanagisawa (1976, 1977), employs cytochemical techniques using an electron microscope to obtain the detailed information on the localization of the phosphatase(s) hydrolyzing the former two of the substrates mentioned above, in the body wall of *A. cantonensis*.

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Materials and Methods

Female *A. cantonensis* were collected from the pulmonary artery and heart of albino rats which were previously infected with the 3rd-stage larvae. Adult females collected were pre-fixed in 0.1 M cacodylate buffer (pH 7.3) containing 2% paraformaldehyde and 1% glutaraldehyde at 4 C for 90 min. Central part of the body (about 3 mm long) was cut in the same solution using a single-edged razor blade, fixed for an additional 1hr at 4 C and washed thoroughly in 0.1 M cacodylate buffer (pH 7.3) containing 6% sucrose. And then the enzyme reaction was performed according to the methods of Go-

mori (1952) and of Wachstein and Meisel (1957) using β-glycerophosphate solution (pH 5.0) and adenosine-5′-monophosphate solution (pH 7.2) as substrate solution respectively. Controls without substrates were run simultaneously. Thereafter the materials were washed in 0.1 M cacodylate buffer (pH 7.3) containing 6% sucrose and post-fixed for 1hr in 1% osmium tetroxide solution. The fixed materials were dehydrated in an ascending series of ethanol solutions and embedded in Epon 812. Sections showing silver and gold interference colours were examined by means of the electron microscope (Hitachi Hu-12A).

Results and Discussion

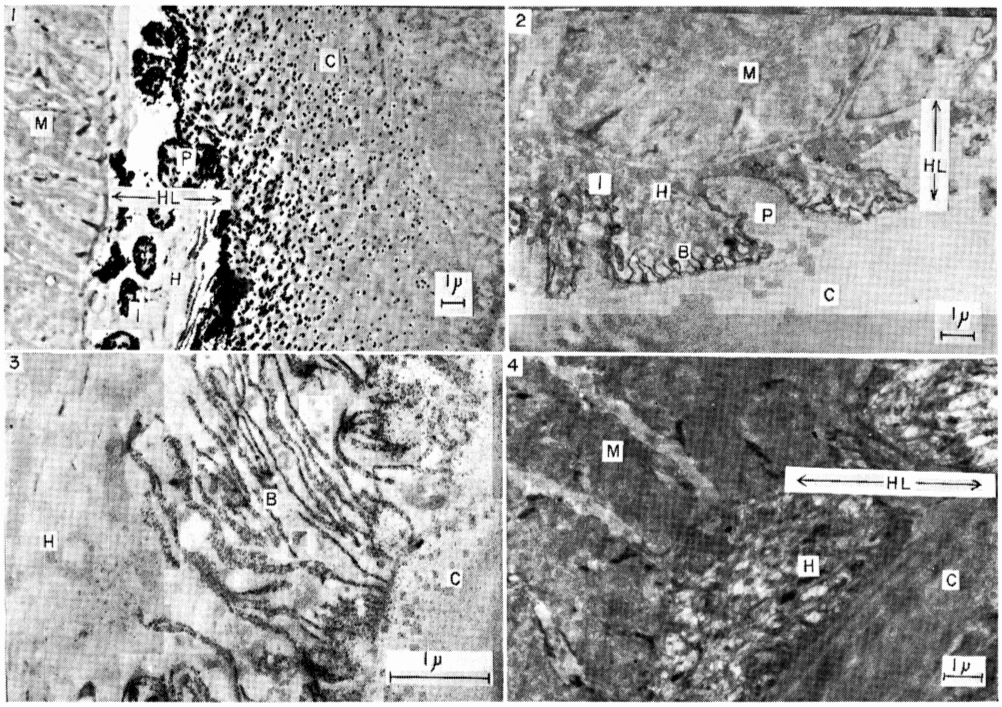
The result using β-glycerophosphate as a substrate at pH 5.0 (Fig. 1) is as follows: Granular deposits of reaction products are seen in the boundary region between the cuticle and the hypodermis. In the cuticle the reaction products are heavier in the inner cuticular portion near the hypodermis than in that near the outer surface of the cuticle. The island-like structures seen in the hypodermal layer show intense phosphatase activity. The peninsula-like structures seen in the hypodermal layer also showed strong phosphatase activity. Contrary to these regions, the somatic musculature exhibits no reaction products.

The localization of a phosphatase hydrolyzing adenosine-5′-monophosphate at pH 7.2 are as follows: Intense reaction products (finer granules) are recognized in the boundary region between the cuticle and hypodermis (Figs. 2, 3). Structures thought to be basal infoldings are discernible in the boundary region between the hypodermis and cuticle and strongly positive for the phosphatase activity (Figs. 2, 3), though such structures are much less obvious in Fig. 1. The enzyme activity in the inner cuticular portion is much higher than that near the outer surface of the cuticle (Fig. 2). In the hypodermal layer the island- and peninsula-like structures as seen in Fig. 2 are fairly positive. In contrast, no enzyme activity is recognizable in the musculature. Throughout the present studies, no deposits is recognizable in the control section (Fig. 4).

In the present studies, the peninsula- and island-like structures in the hypodermal layer and the cuticle near the hypodermis were highly positive for phosphatase activity irrespective of the substrates used. In sections incubated in β-glycerophosphate solution (Fig. 1), however, it was difficult to find out structures comparable to the basal infolding-like structures strongly positive for the enzyme hydrolyzing adenosine-5′-monophosphate (Figs. 2, 3). Whether this reflects the difference in the localization of two phosphatases is unclear. This may be due to the difference of sections examined.

Although the acid phosphatase activity shown histochemically by Yanagisawa *et al.* (1970, 1973) was restricted to the hypodermis of *A. cantonensis*, the present studies reveal the occurrence of the phosphatase(s) which hydrolyze(s) β-glycerophosphate (pH 5.0 and 7.3) and adenosine-5′-monophosphate (pH 7.3) (Maki and Yanagisawa, 1976, 1977) in the cuticle as well. Cuticular phosphatases in nematodes have rarely been reported except those demonstrated by Anya (1966). Moreover it is noteworthy that the acid phosphatase activity in the body wall of *A. cantonensis* is 26.4 times (specific activity) and 3.3 times (total activity) as high as that of the viscera whereas *Ascaris lumbricoides* has much phosphatase in the viscera, especially in its intestine, with the body wall showing very low, if any, activity (Maki and Yanagisawa, 1976, 1977; Yanagisawa and Maki, 1978). At the present time it seems likely that the high phosphatase activity in the body wall of nematodes is characteristic of those parasitic in host body fluid, for instance, *A. cantonensis* in the present study and filarial worms, *Dirofilaria immitis*, *Litomosoides carinii* and *Brugia pahangi* (Yanagisawa and Maki, 1978).

To Anya's thought (1966), the cuticular acid phosphatase cytochemically detectable in the intestinal nematodes is involved in the formation of the cuticle, together with



Explanation of Figures

Fig. 1 Portion of an oblique section of a female *A. cantonensis* showing distribution of an enzyme hydrolyzing β-glycerophosphate in the body wall. Note strong reaction products in the island-like (I) and peninsula-like (P) structures and the cuticle (C) near the hypodermis (H). M, muscle layer; HL, hypodermal layer.

Fig. 2 Portion of an oblique section of the body wall of a female *A. cantonensis* showing distribution of an enzyme hydrolyzing adenosine-5′-monophosphate. Note particularly dense deposits in the basal infolding-like structures (B). The island-like (I) and peninsula-like (P) structures are fairly positive. No activity was observed in the muscle layer (M). C, cuticle; H, hypodermis; HL, hypodermal layer.

Fig. 3 Portion of an oblique section of the body wall of a female *A. cantonensis* (higher magnification) showing distribution of the enzyme hydrolyzing adenosine-5′-monophosphate. Note the copious deposits associated with the basal infolding-like structures (B) as well as the cuticle (C).

Fig. 4 Portion of an oblique control section of the body wall of a female *A. cantonensis*. This control section, incubated without adenosine-5′-monophosphate, shows no reaction product. M, muscle layer; H, hypodermis; HL, hypodermal layer; C, cuticle.

the esterase in the cuticle (Lee, 1961). The physiological role of the phosphatases in the body wall of nematodes dwelling in the host body fluid, for example, *A. cantonensis* and filarial worms mentioned above still remains a matter for conjecture. As far as the present authors observed, the phosphatase localization in the basal infolding-like structures of *A. cantonensis* seems to be closely analogous to that in the infoldings of tubule epithelial cells of mouse kidney (Sasaki and Fishman, 1973). It is interesting to speculate that the phosphatase in the basal infolding-like structures observed in *A. cantonensis* may play a similar role to that in the kidney, assuming the worm phosphatase to be asso-

ciated with the absorption and utilization of glucose through the body wall as suggested by Yanagisawa *et al.* (1970, 1973). Kidney phosphatase has been shown to catalyze the formation of glucose-6-phosphate as well as its hydrolysis and postulated to play a role in the transport of glucose across the kidney tubule cells (Nordlie and Soodsma, 1966). Whether the phosphatase in *A. cantonensis* is concerned in other functions, for instance, protein synthesis and excretory and secretory mechanisms is also a question to be resolved.

Likewise the ultrastructural finding of *Brugia malayi*, a nematode parasitic in host body fluid, reported by Vincent *et al.* (1975), is noteworthy: The regular basal infoldings of lateral chord cell membrane, in close proximity to the multivesicular bodies (generally known to be strongly positive for acid phosphatase) and mitochondria, indicate that in *B. malayi* the outer subcuticular region of the lateral chords is a functional complex which is probably engaged in the exchange of water and solutes across the cuticle. Similarly, basal infolding-like structures (Koyama, personal communication) and mitochondria (Koyama *et al.*, 1972) were observed in the boundary region between the cuticle and the hypodermis of *A. cantonensis* by use of an electron microscope.

The present authors have investigated the enzymological characters and function of the phosphatase in intact *A. cantonensis* and suggested the permeability of the cuticle of the parasite to β-glycerophosphate in the external medium (Maki and Yanagisawa, 1976, 1977). Together with these studies, the present observations lead to the possibility that the phosphatase(s) in question is similar to the ecto-phosphatases as described by DePierre and Karnovsky (1973). The body wall of nematodes, particularly of those inhabiting host body fluid, provides a useful model for further systematical studies from the viewpoints of morphology, histochemistry, cytochemistry and biochemistry.

Summary

Cytochemical studies using the electron microscope were undertaken to clarify the ultrastructural localization of the phosphatase(s) in the body wall of *Angiostrongylus cantonensis*, which hydrolyze(s) β-glycerophosphate (pH 5.0) and adenosine-5′-monophosphate (pH 7.2). Intense reactions were seen in the boundary region between the cuticle and the hypodermis when in use of both substrates. In the cuticle the reaction products were heavier in the inner cuticular portion near the hypodermis than in that near the outer surface of the cuticle, irrespective of substrates used. The island-like and peninsula-like structures seen in the hypodermal layer showed intense reactions for the phosphatase(s) hydrolyzing β-glycerophosphate and adenosine-5′-monophosphate. Structures thought to be basal infoldings in the boundary region between the hypodermal layer and the cuticle were strongly positive for the enzyme hydrolyzing adenosine-5′-monophosphate. Contrariwise, the somatic musculature exhibited no reaction products. The physiological significance of the enzyme in relation to the localization was discussed.

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広東住血線虫の体壁部に於けるフォスファターゼの電顕細胞化学的研究

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広東住血線虫の体壁につき電子顕微鏡による細胞化学的検討を試みて β-グリセロリン酸及びアデノシン-5′-モノリン酸を夫々 pH 5 及び 7.2 で水解するフォスファターゼの局在性を明らかにした.

いずれの基質に於ても以下のような結果が得られた. 反応産物が認められたのは角皮, 角皮と角皮下層の境界近傍及び角皮下層に於てであった. 角皮では体表に近いところよりも, より内側に活性が高い. 角皮下層では島状部分及び半島状の部分に強い活性が観察された. 角皮と角皮下層の境界近傍に basal infolding と思われる構造がみられ, ここにアデノシン-5′-モノリン酸を水解するフォスファターゼ活性が強陽性であった. 以上の部分とは対照的に筋層では全く反応が陰性であった.

本酵素の体壁に於ける局在性と関連してその生理的意義の考察を行った.